

Effects of Dietary Boron in Rats Fed a Vitamin D-deficient Diet

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Although boron has long been known to be a required nutrient for plants, it was not until recently that there was any suggestion of a nutritional requirement for animals and humans. Addition of boron to the diet of vitamin D-deficient chicks indicated that boron may play a role in animal nutrition. Studies with rats have demonstrated that supplemental dietary boron has most marked effects when the diet is deficient in known nutrients. We observed higher apparent-balance values of calcium, magnesium, and phosphorus for rats fed a vitamin D-deprived diet with dietary supplemental boron (2.72 ppm), than for rats fed the same diet without added boron (0.16 ppm). The treatment group with dietary supplemental boron demonstrated a high degree of variability in response to boron. We hypothesize that relatively large and variable vitamin D stores in weanling rats from a colony supplemented with 3000 IU vitamin D/kg diet accounted for the observed variable response. A recent, unpublished study using weanling rats from a low-vitamin D colony appears to support this hypothesis. — *Environ Health Perspect* 102(Suppl.7):55–58 (1994)

Key words: boron, vitamin D, calcium, magnesium, phosphorus, apparent balance, plasma calcium

Introduction

Boron has been known to be a required nutrient for plants since the early part of the 20th century (1). Early studies with dietary boron in rats had equivocal results and further investigation of boron as a required nutrient for animals was ignored (1). It was not until 1981 when Hunt and Nielsen discovered a possible need for boron in the diet of the chick that the study of boron in animals received further interest (2). Hunt and Nielsen observed that vitamin D-deficient chicks responded with improved growth and lower concentrations of serum alkaline phosphatase when boron was added to the diet. Hunt later demonstrated that in the vitamin D-deficient chick, boron decreased body growth but enhanced initiation of cartilage calcification (3). In chicks with concomitant magnesium deficiency, boron had the opposite effect (3). However, most effects of low boron diets were seen when vitamin D in the diet was also low. In growing male rats, Brommage (R. Brommage personal communication,

1989) found no differences in calcium, magnesium, and phosphorus apparent balance as well as serum concentrations of 25-hydroxyvitamin D₃ and 1,25-dihydroxyvitamin D₃ when the only difference between dietary treatment groups was supplemental boron. Although both diets contained adequate vitamin D, it was not clear whether the diet unsupplemented with boron provided a low amount of the element since the diet was never analyzed for boron. Boron has been reported to have an effect in rats when other nutrients such as magnesium, calcium, or methionine are fed in amounts below recommended dietary guidelines (4–7). Researchers in our laboratory recently investigated the effects of supplemental (2.72 ppm) or low (0.16 ppm) dietary boron on rats fed a vitamin D-deprived diet for 12 weeks (8).

Effect of Dietary Boron on Vitamin D-deficient Rats

We fed a total of 19 Harlan Sprague-Dawley (Indianapolis, IN) weanling (21 days old) rats a vitamin D-deprived, acid-washed, corn meal-based diet for 12 weeks (Table 1). Our mineral mix formulation essentially followed that of Nielsen et al. (7), except that we added a higher concentration of potassium because their previous formulation had a concentration below that recommended for rats. Supplemental boron (2.72 ppm-analyzed value) was added to the diet of one treatment group (9 rats), and the other group (10 rats) was fed the diet without supplemental boron (0.16 ppm-

analyzed value). Boron analyses were conducted by Dr. Curtiss Hunt of the United States Department of Agriculture, Agricultural Research Service (USDA, ARS) Human Nutrition Research Center, Grand Forks, ND. Plasma calcium was monitored in three different rat/treatment groups at 3-week intervals (3, 6, and 9 diet weeks) during the study. Apparent balance of calcium, magnesium, and phosphorus was assessed in 13 rats (6 from the supplemental boron

Table 1. Composition of rat diet for initial boron study.

Ingredient	Amount, g/kg dry diet
Casein, high protein ^a	160.00
Ground corn, acid washed ^a	708.00
Corn oil ^b	75.00
Methionine, L ^c	3.00
Mineral mix ^d	42.00
Choline bitartrate ^c	2.00
AIN-76A vitamin mix ^a	10.00

^aUnited States Biochemical Corporation, Cleveland, OH. ^bMazola corn oil. ^cSigma Chemical, St. Louis, MO. ^dMineral mix contained (in g/kg of diet): sodium chloride, 2.0; magnesium acetate, 3.5; manganese acetate, 0.1125; copper sulfate, 0.03; potassium iodide, 0.0004; zinc acetate, 0.05; sodium selenite, 0.0003; ammonium molybdate, 0.004; chromic chloride, 0.002; ammonium vanadate, 0.0003; nickel chloride, 0.002; sodium arsenate, 0.005; potassium chloride, 3.5; potassium acetate, 4.53; sodium metasilicate, 0.05; potassium fluoride, 0.0025; ferrous sulfate, 0.2; calcium phosphate (dibasic), 17; acid-washed ground corn, 11,011. This mineral mix yielded a dietary calcium, magnesium, and phosphorus content of 0.5%, 0.04%, and 0.39% respectively. The boron-supplemented diet contained 0.017 g boric acid/kg diet, to provide 3 mg B/g diet.

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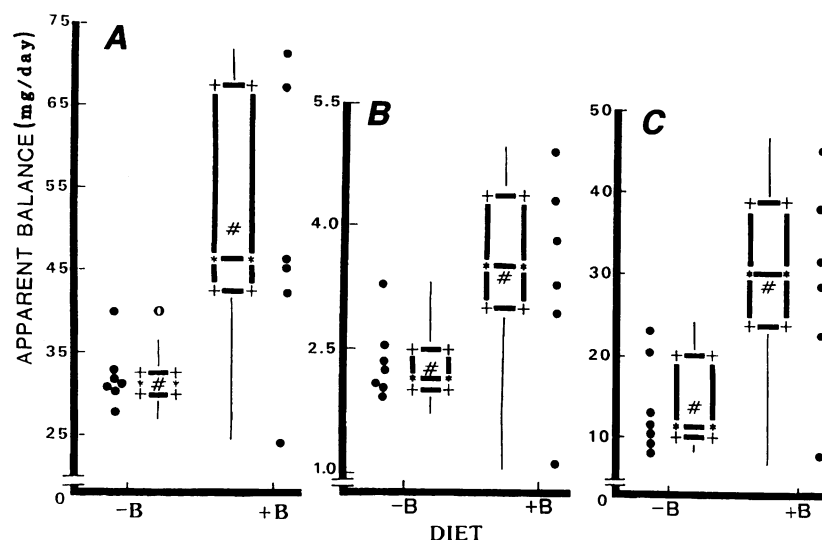


Figure 1. Box plots and individual data points (●) of apparent calcium (a), magnesium (b), and phosphorus (c) balance in vitamin D-deficient rats with (+B) or without (–B) supplemental boron. Univariate procedure of SAS® was used to distribute the data quartiles and indicate the mean (#), median (central horizontal line), and the 25th and 75th percentiles (designated by the lower and upper horizontal lines, respectively). The zero above the extending “whiskers” of the box plot in the first panel indicates that the data point is outside of the 1.5 interquartile range, which is equal to the distance between the 25th and 75th percentiles.

Table 2. Table of significant effects of boron on tissue calcium and phosphorus.^{a,b}

Liver	Treatment ^c	Total P, mg ^d	mg P/g ^e
	2.72	23.6 ± 2.16	2.64 ± 0.23
	0.16	29.8 ± 1.74	3.21 ± 0.10
Cerebellum	Treatment ^c	Total P, mg ^d	mg P/g ^e
	2.72	0.645 ± 0.063	2.78 ± 0.25
	0.16	0.873 ± 0.063	3.48 ± 0.19
Brain cortex	Treatment ^c	Total Ca, mg ^d	mg Ca/g ^e
	2.72	0.053 ± 0.002	0.043 ± 0.007
	0.16	0.082 ± 0.008	0.065 ± 0.000

^aSee Hegsted et al. (8). ^bWet weight basis. ^cAmount of boron in diet per analysis (ppm). ^dBoron effect, $p < 0.05$.

^eBoron effect, $p < 0.02$.

group, 7 from the low boron group) for a 4-day period during week 12 of the study. At the time of sacrifice, the liver, both kidneys, spleen, brain cortex, cerebellum, and brain stem were removed for determination of calcium, magnesium, and phosphorus content. The right anatomical femur was removed for bone analyses, which included density determination; a dry, fat-free weight; ash weight; and calcium, magnesium, and phosphorus content. For further methodologic details see Hegsted et al. (8).

Statistically significant differences were only observed for apparent balance (Figure 1), liver phosphorus, brain cerebellum phosphorus, and brain cortex calcium (Table 2). The apparent decreased balance for calcium, magnesium, and phosphorus in rats that were fed a vitamin D-deprived diet with no supplemental boron reflected mainly a decreased absorption of these minerals from

the diet. The increased concentration of brain cortex calcium and brain cerebellum phosphorus observed in the treatment group that was fed low dietary boron appears to provide a direct measure of changes occurring in brain tissue as a consequence of low dietary boron. Penland has reported changes in electroencephalograms from human subjects who consumed diets low in boron and magnesium (9,10). These effects of low boron intake on brain minerals in rats and brain encephalograms in humans occurred in conjunction with a vitamin D or a magnesium deficiency, respectively. This suggests that brain function could be altered by low dietary boron in combination with other nutrient deficiencies.

A high degree of variability was observed within the boron-supplemented study group for apparent calcium, magnesium, and phosphorus balance, when compared

with the more narrow range in the group with no supplemental boron (Fig. 1). This may have been caused by variable boron requirements of individual rats in a vitamin D-deficient state. Alternatively, variability in vitamin D stores among individual rats may have affected the response to dietary boron. Evidence for the hypothesis that rats in our study had variable vitamin D stores was demonstrated by variable plasma calcium concentrations throughout the study (Figures 2, 3).

Plasma calcium was used in our study as the indicator of vitamin D deficiency. Hypocalcemia is usually associated with vitamin D deficiency in rats (11), especially when amounts of calcium recommended by the National Research Council (12) are fed along with a vitamin D-deficient diet (13). However, recent studies have demonstrated that high dietary calcium and phosphorus can prevent the hypocalcemia normally associated with vitamin D deficiency in rats (14). In addition, high dietary calcium and phosphorus, along with dietary lactose, can also prevent an elevated plasma concentration of parathyroid hormone (15).

The lack of an effect of the dietary treatments on bone and most tissue mineral concentrations may be related to the timing of the measurements. The mineral content of the bone and tissues examined in our study reflected mineral retention over the lifespan of the rats (105 days), including a suckling period with adequate vitamin D and, presumably, ample boron in their mothers' milk. The apparent balance measurements represented the state of the animal during the last four days prior to sacrifice, following 12 weeks on a vitamin D-deprived diet with or without supplemental boron.

Further Studies and Results

The rats used in our study were from a colony supplemented with 3000 IU of vitamin D/kg of diet. We have previously used rats from a low-vitamin D Harlan Sprague-Dawley colony that had no supplemental vitamin D added to their customary, unrefined diet (13). The rats from the low-vitamin D colony had lower vitamin D stores than the rats from the regular (vitamin D-supplemented) Harlan Sprague-Dawley colony. Weanling rats from the low-vitamin D colony exhibited vitamin D deficiency signs earlier when fed a vitamin D-deficient diet (American Institute of Nutrition-76A); higher apparent calcium, magnesium, and phosphorus balance at the end of the study (64–72 days of age) when fed a vitamin D-adequate

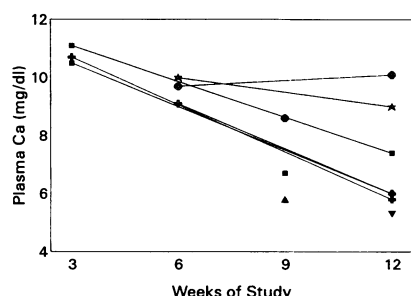


Figure 2. Plasma calcium concentrations during the 12 weeks that rats were fed a vitamin D-deficient, acid-washed, cornmeal-based diet with no supplemental dietary boron. The diet contained (analyzed value) 0.16 ppm (mg/kg diet) of boron. Symbols that are connected represent values from the same rat.

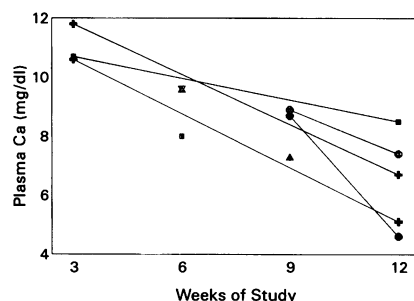


Figure 3. Plasma calcium concentrations during the 12 weeks that rats were fed a vitamin D-deficient, acid-washed, cornmeal-based diet with supplemental boron. The diet contained (analyzed value) 2.72 ppm (mg/kg diet) of boron. Symbols that are connected represent values from the same rat.

diet; and slower initial growth rate, followed by accelerated growth when fed a vitamin D-adequate diet. Therefore, use of low-vitamin D colony rats to investigate the effects of boron may enhance results. Our results from experiments with low-vitamin D and regular colony rats were similar to the results of Halloran (16). He also observed that low-vitamin D-fed rats and regular-diet rat colonies develop hypocalcemia at different rates.

Results from our laboratory support our hypothesis that a delay in depletion of vari-

able vitamin D stores limits the response of rats to dietary boron (17). In the first of two studies with low-vitamin D-fed colony weanling rats, problems with the cornmeal-based diet (fed 1 week), resulted in a switch to rat chow (fed 12 hr) and then to a vitamin D-deficient, purified diet (18). (Table 3; diet is similar to the AIN-76A diet). The calculated value for the amount of boron added to the purified diet was 3 mg/kg diet or 3 ppm. However, the value from the analysis was 1.08 mg/kg diet (analyzed by Dr. Curtiss Hunt). The unsupplemented diet had an analyzed value of 0.23 mg boron/kg diet. Following 5 weeks on this diet, a small but significantly higher total plasma calcium ($p < 0.05$) in the 8-week-old boron-supplemented rats indicated the need for a second study. In the second study, the rats fed supplemental boron had higher total plasma calcium at 8 ($p < 0.001$), 10 ($p < 0.0005$) and 12 ($p < 0.05$) weeks of age. These results suggest that supplemental boron prevents the severe hypocalcemia of vitamin D deficiency. This condition occurs earlier in low-vitamin D-fed colony rats than in vitamin D-supplemented colony rats, during a time period associated with rapid bone growth.

Our most recent results also demonstrated effects of supplemental dietary boron using a purified (17) diet. Diet preparation time and effort are much reduced when feeding animals a purified diet. Although further work is needed to confirm that purified diets provide low boron content, use of a purified diet with an analyzed low-boron content instead of the acid-washed cornmeal-based diet may simplify future rat studies with boron.

Table 3. Purified diet fed to rats from low-vitamin D colony.

Ingredient	Percent
Casein high nitrogen	15.2
DL-methionine	0.3
Cornstarch	32.8
Sucrose	32.8
Fiber-celufil	5.6
Corn oil ^a	8.1
AIN mineral mixture ^b	4.0
AIN-76A vitamin mixture without cholecalciferol ^c	1.0
Choline chloride	0.2

^aBHT was added at 0.01% of corn oil. ^bMineral mixture composition (g/kg): calcium phosphate, dibasic (CaHPO_4), 500; sodium chloride, 74; potassium citrate monohydrate, 220; potassium sulfate (K_2SO_4), 52; magnesium oxide (MgO), 24; manganese carbonate (MnCO_3) (43–48% Mn), 3.5; ferric citrate (16–17% Fe), 6; zinc carbonate (70% ZnO), 1.6; cupric carbonate (53–55% Cu), 0.3; KIO_3 , 0.01; sodium selenite, 0.01; chromium potassium sulfate, 0.55. ^cVitamin mixture composition (mg/kg): thiamine HCl, 600; riboflavin, 600; pyridoxine HCl, 700; nicotinic acid, 3000; D-calcium pantothenate, 1600; folic acid, 200; D-biotin, 20; cyanocobalamin, 1; retinyl palmitate premix (500,000 IU/g), 1; DL- α -tocopheryl acetate premix (250 IU/g), 20,000; menadione (vitamin K), 50.

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